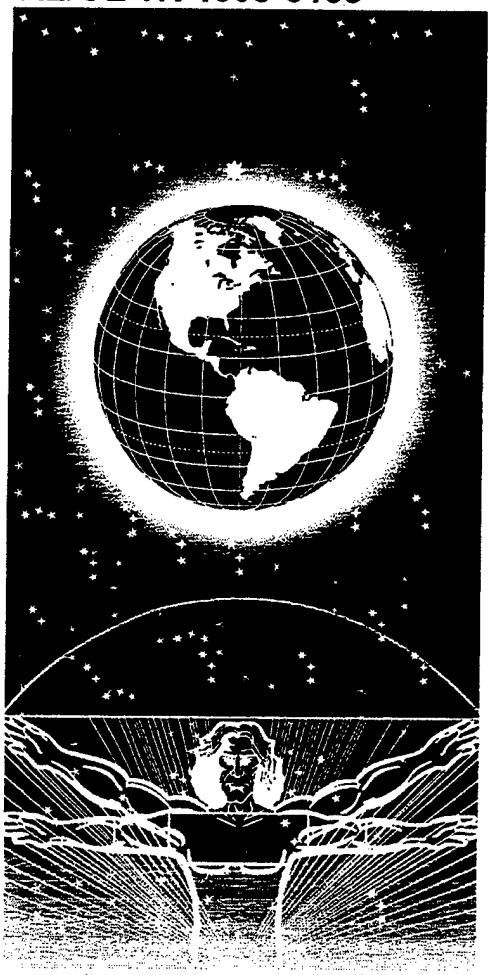
AL/OE-TR-1995-0133



UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

ALTERATION IN NEUROTRANSMITTERS AND THEIR METABOLITE LEVELS IN 1,3,5-TRINITROBENZENE-TREATED SPRAGUE-DAWLEY RATS

Latha Narayanan
Daniel J. Caldwell

GEO-CENTERS, INC.
7 WELLS AVENUE
NEWTON CENTRE MA 02159

Clay R. Miller

US ARMY MEDICAL RESEARCH DETACHMENT
WALTER REED ARMY INSTITUTE OF RESEARCH
WRIGHT-PATTERSON AFB OH 45433-7400

April 1995

Occupational and Environmental Health Directorate
Toxicology Division
2856 G Street
Wright-Patterson AFB OH 45433-7400

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Air Force Armstrong Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Service
8725 John J. Kingman Rd., Ste 0944
Ft. Belvoir, Virginia 22060-6218

DISCLAIMER

This Technical Report is published as received and has not
been edited by the Technical Editing Staff of the Air Force Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1995-0133

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

STEPHEN R. CHANNEL, Maj, USAF, BSC
Branch Chief, Operational Toxicology Branch
Air Force Armstrong Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1995	Interim Report - September 1993-June 1994

4. TITLE AND SUBTITLE
Alteration in Neurotransmitters and their Metabolite Levels in 1,3,5-Trinitrobenzene-Treated Sprague-Dawley Rats

5. FUNDING NUMBERS
Contract N00014-95-D-0048
PE 62202F
PR 7757
TA 7757A1
WU 7757A105

6. AUTHOR(S)
L. Narayanan, D. Caldwell, and C. Miller

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Man Tech Geo-Centers Joint Venture
P.O. Box 31009
Dayton, OH 45437-0009

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Armstrong Laboratory, Occupational and Environmental Health Directorate
Toxicology Division, Human Systems Center
Air Force Materiel Command
Wright-Patterson AFB, OH 45433-7400

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
AL/OE-TR-1995-0133

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

1,3,5-trinitrobenzene (TNB), a class-A explosive, is an anthropogenic environmental contaminant found in effluent released from facilities that synthesize, produce or demilitarize munitions, or in the leachate from disposal of solid 2,4,6-trinitrotoluene (TNT) wastes. We have reported that rats exposed to TNB show signs of neurological stress (head tilting, loss of equilibrium and "cork screw" like motion) and bilateral lesions in medulla oblongata and cerebral peduncle. The underlying biochemical mechanism(s) for TNB-induced neurological damage in rats is not known. We analyzed brain tissue for neurotransmitters and their metabolites in control and TNB treated rats using HPLC and electrochemical detection in nine different brain regions. We found statistically significant increases in: a) no-epinephrine levels in all regions except frontal cortex; b) epinephrine levels in brain stem, septum and cerebellum; c) 5-HT levels in thalamus, hypothalamus, frontal cortex, caudate nucleus and cerebellum; d) dopamine levels in thalamus, brain stem, and hypothalamus. Significant decreases in dopamine levels were observed in caudate nucleus and septum. Change in neurotransmitter levels in a specific region or regions may be one possible mechanism for TNB-induced neurological damage.

14. SUBJECT TERMS
1,3,5-trinitrobenzene (TNB)
Neurotransmitters
Neurological stress
Mechanism for TNB-induced neurological damage

15. NUMBER OF PAGES
46

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

THIS PAGE INTENTIONALLY LEFT BLANK

PREFACE

There is an inadequate data base for use in establishing a reference dose (RfD) for 1,3,5-TNB. There is little or no toxicity data in IRIS, which also results in a large uncertainty factor applied to the "confidence" of the resulting RfD. The RfD will be used to set cleanup standards for remediation of contaminated soil and water.

Since 1,3,5-TNB is not carcinogenic, the Hazard Index (HI) is used to determine the requirement for remediation. This index is calculated from the RfD, which in turn is calculated from the no observed adverse effect level (NOAEL) derived from toxicity studies and the default uncertainty factors (UF) employed by the US EPA. Currently, TNB is regulated based on its structural similarity to 1,3-DNB, which is more acutely toxic (i.e., lower LD_{50} value). The current RfD for 1,3,5-TNB is also based on structural similarity to 1,3-DNB and the UF is adjusted for this assumption. The results is the greatest UF permissible (i.e., 10,000).

If the HI can be lowered by a factor of 10 (from 5 to 0.5), soil composting costs decrease by an order of magnitude from $14M to $1.5M. The cost of ground water remediation is as yet unknown; however, the RPM is discussing pump and treat clean up methods which will be more economical if the HI is lowered.

Results of a recent study conducted to evaluate the toxicity of TNB (Kinkead et al, 1994a and 1994b) indicated that, at high doses, TNB caused neurological effects in treated rats. This report presents results of neurotransmitter and metabolite analysis of brain tissue from TNB treated rats.

TABLE OF CONTENTS

LIST OF BARGRAPHS

LIST OF TABLES

LIST OF FIGURES

FIGURE **PAGE**

INTRODUCTION

The dimorphic crystalline solid 1,3,5-trinitrobenzene (TNB) is a Class-A explosive that is less sensitive to impact but more powerful than 2,4,6-trinitrotoluene (TNT). Exposure to TNB, an anthropogenic environmental contaminant, can occur through contact with water effluents released from facilities that synthesize, produce or demilitarize munitions or from the disposal of solid TNT wastes (Ryon *et al*, 1984; U.S. EPA, 1989). Previous reports from this laboratory indicate that rats exposed to TNB show signs of neurological disorders such as head tilting, loss of equilibrium and "cork-screw"-like motion. Bilateral lesions in the medulla oblongata and cerebral peduncle were observed histologically (Kinkead et al, 1994a and 1994b). The underlying biochemical mechanism(s) of neurological disorders induced by TNB in rats is not known.

In this study, the neurotransmitters norepinephrine (NE), epinephrine (E), dopamine (DA), 5-hydroxy-triptamine (5-HT) and the metabolites in control and TNB-exposed rats were analyzed using HPLC coupled with electrochemical detection from nine brain regions. Dopamine's metabolite, homovanillic acid (HVA) and 5-HT's metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA) were analyzed.

MATERIALS AND METHODS

Test Agent and Doses

The TNB/diet mixture was provided by the U.S.Army through a contract with the Environmental Protection Agency. Pertinent chemical and physical properties of the test compound are listed below:

1,3,5-trinitrobenzene(TNB)

Synonyms:	Trinitrobenzene
	Benzenite
CAS#:	99-35-4
Empirical Formula:	$C_6H_3N_3O_6$
Formula Weight:	213.11
Vapor Pressure:	3.2×10^{-6} mmHg at 20°C

Male and female Sprague-Dawley-derived outbred albino rats, known as Charles River CD rats, were purchased from Charles River Breeding Laboratories, Raleigh, NC. The TNB was mixed appropriately in the diet and administered orally. The target dose was in the range 0 to 800 mg TNB/kg diet. Varied food consumption rate resulted in the male rats receiving approximately 51, 23, and 3 mg TNB/kg body weight/day in the high-, mid-, and low-dose groups, respectively. The female rats received 60, 30, and 4mg TNB/kg body weight/day. Female rats were exposed to TNB for 90 days. Minimum exposure to TNB in male rats was 28 days, since the objective of the range-finding study, in coordination with this neurotransmitter study, was to determine the dose levels to be used in a 90-day modified Screening Information Data Set (SIDS) protocol to address the developmental and reproductive toxicity of TNB in rats.

Materials

The materials used in this study and their sources are:

Sigma (St. Louis, MO):
NE bitartrate (A-9152)
DA hydrochloride (H-8502)
5-HT creatinine sulfate complex (H-7752)
5-HIAA (H-8876)
Epinephrine
3,4-dihydroxybenzylamine hydrobromide (DHBA, D-7012)
HVA (NO 1252)
DOPAC (D-9128)
Sodium phosphate (S-0751)

Eastman-Kodak (Rochester, NY):
Citric acid (A-940)
Sodium octyl sulfate (No. 10577)

Aldrich (Milwaukee, WI):
Citric acid (A-940)
Disodiumethylenediaminetetraacetic acid (EDTA, 10, 631-3)

Fisher (Fairlawn, NJ):
Perchloric acid 70% (UN-1873)

Sigma-Aldrich, Sigma (St. Louis, MO); Aldrich (Milwaukee, WI):
Methanol (HPLC grade, 27, 047-4)

Water was deionized and glass distilled.

HPLC apparatus

HPLC determinations were performed with Dionex Model, DX-300 isocratic liquid chromotograph coupled with a pulse electrochemical detector (PED-2). An advanced gradient pump (AGP-Standard size) was used. A glassy-carbon working electrode was set at 0.8 V vs a Ag/Agcl reference electrode. The sensitivity of the detector was maintained between 0.5 and 1.0 nA depending on the concentration of the neurotransmitters. Separation by isocratic elution was performed on C_{18}, reverse phase column, preceded by a guard column (Guard-Pak, C_{18} Waters Association, Milford, MA).

Mobile Phase

The mobile phase was 15%(v/v) methanol in a solution (pH 4.2) of 32 mM citric acid, 12.5 mM disodium hydrogen orthophosphate, 0.5 mM octyl sodium sulfate and 0.05 mM EDTA. The mobile phase was filtered through a 0.45-μm filter (Millipore, Bedford, MA) and then degassed under vacuum before use. A flow rate of 1.2 mL/min(2200 p.s.i) at ambient temperature was employed in this study.

Standard curve

Known amounts of NE, DA, E, 5-HT, DOPAC, HVA and 5-HIAA, in the range 0.2-20 ng were injected into the HPLC system. DHBA (2.5 ng) was used as internal standard. All compounds were easily oxidized at 0.8 V vs a Ag/Agcl reference electrode. Each of these compounds gave a linear response in the range (0.2-20 ng).

Animal Study

Control and TNB-administered rats were euthanized by carbon dioxide inhalation. The brains were surgically removed, and nine regions of the brains were dissected, frozen on dry ice, and stored at -70°C until assayed. The nine brain regions were the brainstem; frontal cortex; cerebral cortex; caudate nucleus; septum; hypothalamus; thalamus; hippocampus; and cerebellum.

The samples were thawed and homogenized for 30 sec. in 0.17M Perchloric acid (90mg tissue per 1.0mL of 0.17M perchloric acid), containing 125ng of DHBA as an internal standard. A polytron homogenizer was used for homogenization. Homogenates were centrifuged at 4°C for 30 min. at 31500g. The supernatants were separated and immediately analyzed by injecting 20μl of supernatants into the HPLC system using the autosampler.

Levels of neurotransmitters and their metabolites in nine regions of the brain in control and TNB exposed rats were thus measured.

RESULTS

Representative chromatograms obtained for NE, E, DA, 5-HT, DOPAC, HVA and 5-HIAA standards in 0.17M perchloric acid are shown in (Figure 1). Levels of neurotransmitters and their metabolites in nine brain regions of control and TNB-dosed (low-, mid-, and high-dosed) female and male rats were quantitated using standard curves (TABLES 1-14).

Statistically significant changes in the neurotransmitter levels in TNB-treated groups compared to control group were calculated using a student t-test(for unpaired data). Significant changes in NE, E, 5-HT and DA levels in TNB-exposed rats compared to control rats are depicted by representing data in Bargraphs (Bargraphs 1-12).

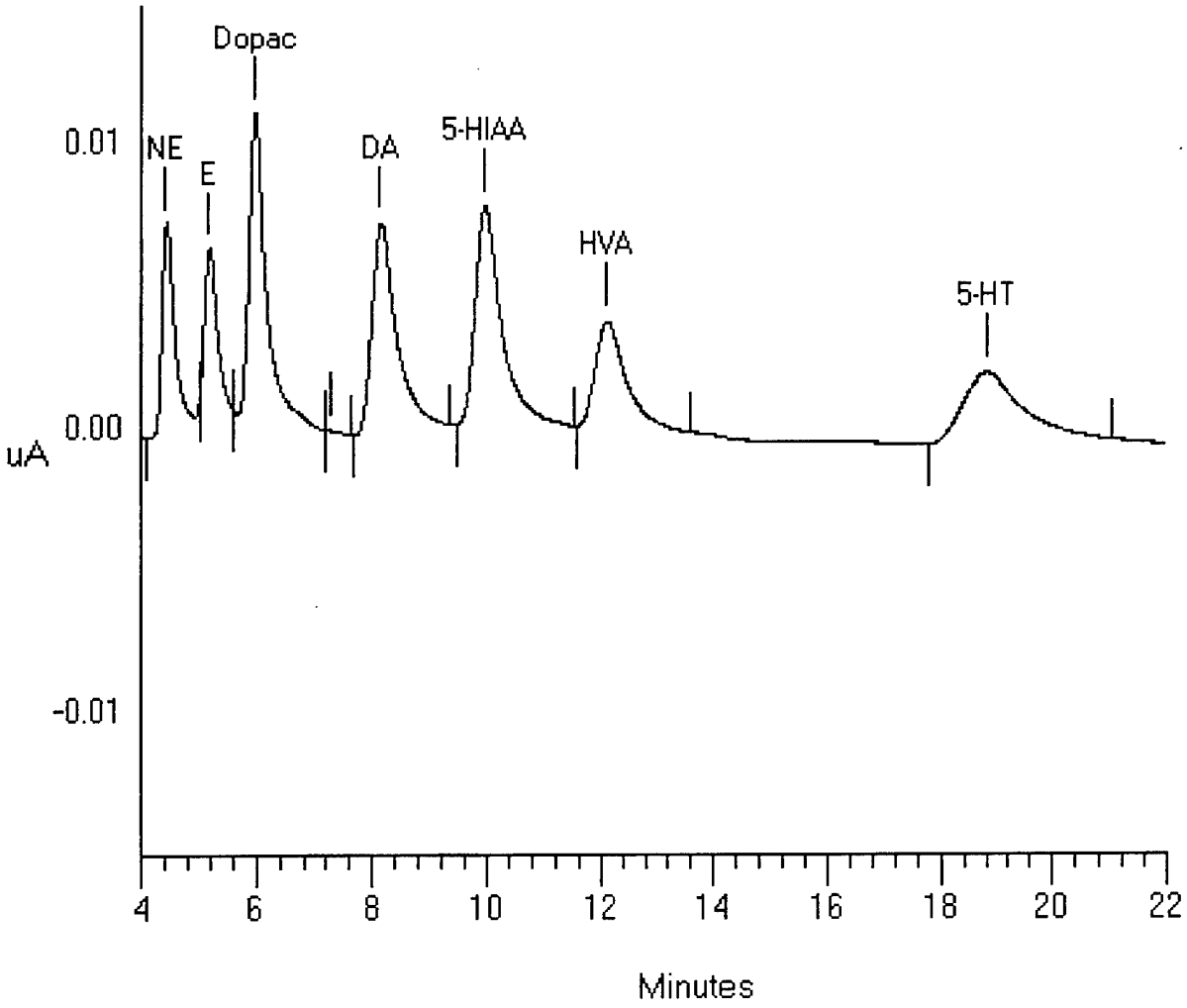


FIGURE 1. Neurotransmitter's elution pattern

TABLE 1. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON NOREPINEPHRINE (NE) LEVELS IN NINE REGIONS OF BRAIN IN FEMALE RATS

Brain regions	NE conc in μgm/g wet weight in control rats.	NE conc in μgm/g wet weight (low-dose) and % change from control	NE conc in μgm/g wet weight (Mid-dose) and %-change-from control	NE conc in μgm/g wet weight (high-dose) and %-change from control.
Septum	7.3	88.45	130.7	136.5
		(1111.6%)	(1690.4%)	(1769.7%)
Brainstem	8.28	18.91	20.52	43.59
		(128.4%)	(147.8%)	(426.5%)
Cerebellum	5.98	32.37	38.1	38.16
		(441.3%)	(537.4%)	(538.1%)
Frontal Cortex	8.39	9.39	11.14	11.33
		(11.92%)*	(32.75%)*	(35.04%)*
Cerebral Cortex	10	15.76	19.92	20.55
		(57.6)	(99.17%)	(105.5%)
Caudate nucleus	2.14	4.13	4.76	6.14
		(93.1%)	(122.4%)	(187.1%)
Thalamus	5.42	5.69	6.64	6.7
		(5.06%)*	(22.41%)	(23.3%)
Hypothalamus	6.06	8.3	16.79	24.99
		(36.98%)	(177.1%)	(312.4%)
Hippocampus	2.66	11.52	12.17	16.05
		(333.1%)	(357.5%)	(503.4%)

Note: Each value represents mean for 6 rats (conc., in μg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data).

TABLE 2. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON NOREPINEPHRINE (NE) LEVELS IN NINE REGIONS OF BRAIN IN MALE RATS

Brain regions	NE conc in μgm/g wet weight in control rats.	NE conc in μgm/g wet weight (low-dose) and %-change from control	NE conc in μgm/g wet weight (Mid-dose) and %-change from control.	NE conc in μgm/g wet weight (high-dose) and %-change from control
Septum	5.214	61.85	91.4	95.45
		(1086.2%)	(1652.8%)	(1730.5%)
Brainstem	5.912	13.224	14.35	30.48
		(123.6%)	(142.8%)	(415.5%)
Cerebellum	4.271	22.61	26.64	26.69
		(429.4%)	(523.7%)	(524.7%)
Frontal Cortex	5.993	6.566	7.79	7.92
		(9.57%)*	(29.96%)*	(32.21%)*
Cerebral Cortex	7.143	11.02	13.93	14.37
		(54.2%)	(95.02%)	(101.2%)
Claud Nucleus	1.529	2.89	3.33	4.29
		(89.08%)	(117.7%)	(180.9%)
Thalamus	3.87	3.98	4.64	4.8
		(2.85%)*	(19.82%)	(24.05%)
Hypothalamus	4.329	5.85	11.74	17.85
		(35.45%)	(173.4%)	(312.3%)
Hippocampus	1.9	8.057	8.51	11.22
		(324.1%)	(347.9%)	(490.7%)

Note: Each value represents mean for 6 rats (conc., in μg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data).

TABLE 3. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON EPINEPHRINE (E) LEVELS IN NINE REGIONS OF BRAIN IN FEMALE RATS

Brain regions	E conc in μgm/g wet weight in control rats.	E conc in μgm/g wet weight (low-dose) and %-change from control.	E conc in μgm/g wet weight (Mid-dose) and %-change from control	E conc in μgm/g wet weight (high-dose) and %-change from control
Septum	19.95	49.13	62.84	70.06
		(146.3%)	(214.9%)	(251.2%)
Brainstem	0.7	0.79	1.12	1.55
		(12.85%)*	(60.43%)	(121.4%)
Cerebellum	1.87	1.879	2.5	3.63
		(0.48%)*	(33.69%)	(94.12%)
Frontal Cortex	3.72	3.877	4.2	4.27
		(4.2%)*	(12.9%)*	(14.78%)*
Cerebral Cortex	1.79	1.808	1.9	2.41
		(1.01%)*	(6.15%)*	(23.59%)*
Caudate nucleus	12.21	11.965	13.76	13.91
		(-2.01%)*	(12.69%)*	(13.90%)*
Thalamus	2.1	2.2	3.04	2.92
		(4.62%)*	(44.47%)*	(38.71%)*
Hypothalamus	2.14	2.76	2.63	2.69
		(28.97%)*	(22.80%)*	(25.7%)*
Hippocampus	2.2	2.59	2.64	2.68
		(-64.0%)*	(19.80%)*	(21.82%)*

Note: Each value represents mean for 6 rats (conc., in μg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data).

TABLE 4. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON EPINEPHRINE (E) LEVELS IN NINE REGIONS OF BRAIN IN MALE RATS

Brain regions	E conc in µgm/g wet weight in control rats.	E conc in µgm/g wet weight (low-dose) and %-change from control	E conc in µgm/g wet weight (Mid-dose) and %-change from control	E conc in µgm/g wet weight (high-dose) and %-change from control.
Septum	14.25	34.35	43.94	48.99
		(141.1%)	(208.4%)	(243.8%)
Brainstem	0.5	0.553	0.78	1.09
		(4.34%)*	(56%)	(118.88%)
Cerebellum	1.336	1.35	1.75	2.54
		(1.05%)*	(30.89%)	(90.05%)
Frontal Cortex	2.657	2.711	2.94	2.99
		(2.03%)*	(10.53%)*	(12.38%)*
Cerebral Cortex	1.279	1.264	1.33	1.69
		(-1.11%)*	(3.92%)*	(31.81%)*
Caudate nucleus	8.71	8.367	9.62	9.73
		(-4.06%)*	(10.33%)*	(11.81%)*
Thalamus	1	1.538	2.12	2.04
		(2.42%)*	(41.44%)*	(35.80%)*
Hypothalamus	1.529	1.93	1.84	1.92
		(27.77%)*	(22.33%)*	(25.16%)*
Hippocampus	1.571	1.811	1.84	1.87
		(15.26%)*	(17.30%)*	(19.26%)*

Note:- Each value represents mean for 6 rats (conc., in µg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

TABLE 5. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON DOPAMINE (DA) LEVELS IN NINE REGIONS OF BRAIN IN FEMALE RATS

Brain regions	DA conc in μgm/g wet weight in control rats.	DA conc in μgm/g wet weight (low-dose) and % change from control	DA conc in μgm/g wet weight (Mid-dose) and %-change from control	DA conc in μgm/g wet weight (high-dose) and %-change from control
Septum	19.352	16.4	20.47	15.86
		(-15.25%)*	(5.78%)*	(-18.04%)*
Brainstem	8.99	10.67	10.8	17.07
		(18.69%)	(20.13%)	(89.88%)
Cerebellum	14.59	10.142	12.73	15.33
		(-30.49%)*	(-12.76%)*	(5.07%)*
Frontal Cortex	13.08	11.921	12.73	12.08
		(-8.86%)*	(-2.68%)*	(-7.65%)*
Cerebral Cortex	11.46	4.73	12.73	11.7
		(-58.73%)*	(11.08%)*	(2.09%)*
Caudate nucleus	15.16	14.03	13.1	13.81
		(-7.45%)*	(-13.72%)*	(-8.92%)*
Thalamus	9.79	11.01	11.71	15.57
		(12.5%)	(19.7%)	(59.08%)
Hypothalamus	7.23	9.45	12.33	12.39
		(30.71%)	(70.47%)	(71.37%)
Hippocampus	10.83	9.2	9.92	10.2
		(-54.63%)*	(-8.4%)*	(-5.82%)*

Note: Each value represents mean for 6 rats (conc., in μg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

TABLE 6. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON DOPAMINE (DA) LEVELS IN NINE REGIONS OF BRAIN IN MALE RATS

Brain regions	DA conc in μgm/g wet weight in control rats.	DA conc in μgm/g wet weight (low-dose) and %-change from control.	DA conc in μgm/g wet weight (Mid-dose) and %-change from control	DA conc in μgm/g wet weight (high-dose) and %-change from control
Septum	13.823	11.469	14.31	11.09
		(-17.03%)*	(3.56%)*	(-19.76%)*
Brainstem	6.419	7.462	7.55	11.94
		(16.20%)*	(17.60%)*	(86.00%)
Cerebellum	10.421	7.092	8.9	10.72
		(-31.94%)*	(-14.59%)*	(2.87%)*
Frontal Cortex	9.343	8.336	8.9	8.45
		(-10.77%)*	(-4.72%)*	(-9.58%)*
Cerebral Cortex	8.186	3.308	8.9	8.18
		(-59.59%)*	(8.75%)*	(-0.05%)*
Caudate nucleus	10.829	9.811	9.16	9.66
		(-9.40%)*	(-15.43%)	(-10.83%)
Thalamus	6.99	7.699	8.19	10.9
		(10.1%)	(17.1%)	(55.74%)
Hypothalamus	5.164	6.67	8.7	8.85
		(29.43%)	(68.99%)	(71.3%)
Hippocampus	7.736	6.434	6.94	7.13
		(-16.83%)*	(-10.32%)*	(-7.79%)*

Note: Each value represents mean for 6 rats (conc., in μg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

TABLE 7. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON 5-HYDROXYTRIPTAMINE (5-HT) LEVELS IN NINE REGIONS OF BRAIN IN FEMALE RATS

Brain regions	5-HT conc in µgm/g wet weight in control rats.	5-HT conc in µgm/g wet weight (low-dose) and %-change from control.	5-HT conc in µgm/g wet weight (Mid-dose) and %-change from control.	5-HT conc in µgm/g wet weight (high-dose) and %-change from control.
Septum	3.24	2.172	1.02	0.66
		(-32.96%)*	(-68.52%)*	(-79.63%)*
Brainstem	1.23	0.823	0.44	0.49
		(-33.09%)*	(-64.07%)*	(-60.16%)*
Cerebellum	0.02	0.106	0.15	0.18
		(430.04%)	(653.5%)	(800%)
Frontal Cortex	3.37	3.5	3.8	5.45
		(3.86%)*	(12.8%)	(61.7%)
Cerebral Cortex	0.38	0.3846	0.42	0.43
		(1.21%)*	(11.05%)*	(13.16%)*
Caudate nucleus	0.36	0.875	0.89	1
		(143.1%)	(147.2%)	(177.8%)
Thalamus	0.4	0.787	1.56	1.63
		(99.2%)	(294.9%)	(313.1%)
Hypothalamus	0.06	0.56	1.2	1.65
		(833.3%)	(1901.7%)	(2650%)
Hippocampus	5.06	4.358	2.59	4.53
		(-13.87%)*	(-48.85%)*	(-10.47%)*

Note: Each value represents mean for 6 rats (conc., in µg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

TABLE 8. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON 5-HYDROXYTRIPTAMINE (5-HT) LEVELS IN NINE REGIONS OF BRAIN IN MALE RATS

Brain regions	5-HT conc in μgm/g wet weight in control rats.	5-HT conc in μgm/g wet weight (low-dose) and %-change from control.	5-HT conc in μgm/g wet weight (Mid-dose) and %-change from control.	5-HT conc in μgm/g wet weight (high-dose) and %-change from control
Septum	2.314	1.519	0.71	0.46
		(-34.37%)*	(-69.18%)*	(-80.06%)*
Brainstem	0.878	0.576	0.31	0.34
		(-32.40%)*	(-62.72%)*	(-58.90%)*
Cerebellum	0.02	0.106	0.15	0.18
		(430%)	(653.5%)	(800.0%)
Frontal	2.407	2.448	2.66	3.81
		(1.68%)*	(10.4%)	(58.3%)
Cerebral Cortex	0.27	0.269	0.3	0.3
		(-0.91%)*	(10.70%)*	(10.70%)*
Caudate nucleus	0.257	0.612	0.62	0.7
		(138%)	(142%)	(172%)
Thalamus	0.28	0.55	1.09	1.14
		(95.06%)	(286.7%)	(304.5%)
Hypothalamus	0.043	0.45	0.84	1.18
		(946.5%)	(1853.4%)	(2644.1%)
Hippoocampus	3.614	3.048	1.81	3.17
		(-15.68%)*	(-49.93%)*	(-12.35%)*

Note: Each value represents mean for 6 rats (conc., in μg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

TABLE 9. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON 3,4-DIHYDROXYINDOLOACETIC ACID (DOPAC) LEVELS IN NINE REGIONS OF BRAIN IN FEMALE RATS

Brain regions	Dopac conc in µg/g wet weight in control rats.	Dopac conc in µgm/g wet weight (low-dose) and %-change from control.	Dopac conc in µg/g wet weight (Mid-dose) and %-change from control.	Dopac conc in µgm/g wet weight (high-dose) and %-change from control.
Septum	7.85	8.61	9.48	19.29
		(9.68%)*	(20.790%)*	(145.73%)
Brainstem	0.54	0.49	0.24	0.14
		(-9.26%)*	(-54.81%)*	(-74.07%)*
Cerebellum	0.7	0.562	0.55	0.51
		(-19.76%)*	(-21.71%)*	(-27.14%)*
Frontal Cortex	0.35	0.412	0.63	0.99
		(17.71%)*	(79.170%)*	(182.86%)
Cerebral Cortex	0.87	0.517	2.07	1.57
		(-40.57%)*	(137.82%)	(80.46%)*
Caudate nucleus	4.94	4.188	4.86	3.98
		(-15.22%)*	(-1.54%)*	(-19.46%)*
Thalamus	0.62	0.3036	0.33	0.35
		(-50.80%)*	(-47.17%)*	(-43.28%)*
Hypothalamus	1.32	0.394	0.42	0.61
		(-70.15%)*	(-68.48%)*	(-53.79%)*
Hippocampus	0.05	0.018	0.02	0.03
		(-64.00%)*	(-52.6%)*	(-40.00%)*

Note: Each value represents mean for 6 rats (conc., in µg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

TABLE 10. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON 3,4-DIHYDROXYINDOLOACETIC ACID (DOPAC) LEVELS IN NINE REGIONS OF BRAIN IN MALE RATS

Brain regions	Dopac conc in μgm/g wet weight in control rats.	Dopac conc in μgm/g wet weight (low-dose) and %-change from control.	Dopac conc in μgm/g wet weight (Mid-dose and %-change from control.	Dopac conc in μgm/g wet weight (high-dose) and %-change from control.
Septum	5.607	6.021	6.63	13.49
		(7.38%)*	(18.25%)*	(140.58%)
Brainstem	0.386	0.343	0.17	0.1
		(-9.07%)*	(-53.67%)*	(-72.52%)*
Cerebellum	0.5	0.393	0.38	0.36
		(-21.40%)*	(-23.36%)*	(-28.67%)*
Frontal Cortex	0.25	0.288	0.44	0.69
		(15.24%)*	(75.94%)*	(176.92%)
Cerebral Cortex	0.621	0.362	1.45	1.1
		(-41.82%)*	(132.83%)	(76.67%)*
Caudate nucleus	3.529	2.929	3.4	2.78
		(-17.00%)*	(3.60%)*	(-21.15%)*
Thalamus	0.44	0.212	0.23	0.24
		(-51.83%)*	(-48.28%)*	(-44.47%)*
Hypothalamus	0.943	0.276	0.29	0.44
		(-67.24%)*	(-67.05%)*	(-52.66%)*
Hippocampus	0.036	0.013	0.02	0.02
		(-64.76%)*	(-53.59%)*	(-41.26%)*

Note: Each value represents mean for 6 rats (conc., in μg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

TABLE 11. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON HOMOVANILLIC ACID (HVA) LEVELS IN NINE REGIONS OF BRAIN IN FEMALE RATS

Brain regions	HVA conc in µgm/g wet weight in control rats .	HVA conc in µgm/g wet weight in TNB (low-dose) dosed rats.	HVA conc in µgm/g wet weight in TNB (Mid-dose) and %-change from control.	HVA conc in µgm/g wet weight (high-dose) and %-change from control.
Septum	1.847	2.324	3	3.37
		(25.83%)*	(62.43%)*	(82.46%)*
Brainstem	1.15	2.613	3.15	3.26
		(127.2%)	(173.5%)	(183.5%)
Cerebellum	1.6	1.638	3.91	6.23
		(2.37%)*	(144.625%)	(289.38%)
Frontal Cortex	1.25	3.88	3.86	3.6
		(210.48%)	(208.48%)	(188.00%)
Cerebral Cortex	0.93	1.0064	4.12	4.75
		(8.22%)*	(343.4%)	(410.8%)
Caudate nucleus	1.18	1.308	2.4	1.19
		(10.85%)*	(103.47%)	(1.03%)*
Thalamus	0.29	1.8215	3.45	3.8
		(536.89%)	(1106.99%)	(1229.93%)
Hypothalamus	0.05	0.304	0.53	0.41
		(508.00)%	(952.00%)	(720.00%)
Hippocampus	0.97	0.93	1.2	0.85
		(-4.11%)*	(24.120%)*	(-12.37%)*

Note: Each value represents mean for 6 rats (conc., in µg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data).

TABLE 12. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON HOMOVANILLIC ACID (HVA) LEVELS IN NINE REGIONS OF BRAIN IN MALE RATS

Brain regions	HVA conc in µgm/g wet weight in control rats.	HVA conc in µgm/g wet weight in TNB (low-dose) and %-change. from control	HVA conc in µgm/g wet weight in TNB (Mid-dose) and %-change from control	HVA conc in µg/g wet weight in TNB (high-dose) and % change fr0m control
Septum	1.319	1.625	2.1	2.36
		(23.19%)*	(59.02%)*	(78.63%)*
Brainstem	0.821	1.827	2.2	2.28
		(124.53%)	(169.8%)	(179.6%)
Cerebellum	1.143	1.145	2.74	4.36
		(0.23%)*	(139.49%)	(281.21%)
Frontal Cortex	3.37	3.5	3.8	5.45
		(3.86%)*	(12.8%)	(61.7%)
Cerebral Cortex	0.664	0.704	2.88	3.32
		(5.94%)*	(334.1%)	(400%)
Caudate nucleus	0.843	0.915	1.68	0.83
		(138%)	(142%)	(172%)
Thalamus	0.2	1.274	2.41	2.66
		(523.53%)	(1081.67%)	(1202.03%)
Hypothalamus	0.036	0.213	0.37	0.29
		(486.94%)	(923.05%)	(704.91%)
Hippocampus	0.693	0.65	0.84	0.59
		(-6.13%)*	(21.52%)*	(-14.21%)*

Note: Each value represents mean for 6 rats (conc., in µg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data).

TABLE 13. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON 5-HYDROXYINDOLACETIC ACID (5-HIAA) LEVELS IN NINE REGIONS OF BRAIN IN FEMALE RATS

Brain regions	5-HIAA conc in μgm/g wet weight in control rats.	5-HIAA conc in μgm/g wet weight (low-dose) and %-change from control.	5-HIAA conc in μgm/g wet weight (Mid-dose) and %-change from control.	5-HIAA conc in μgm/g wet weight (high-dose) and %-change from control.
Septum	8.245	8.945	10.71	14.58
		(8.49%)*	(29.920%)*	(76.83%)*
Brainstem	3.67	2.89	3.39	5.31
		(-21.25%)*	(-7.57%)*	(44.69%)*
Cerebellum	5.98	2.883	2.28	4.03
		(-51.79%)*	(-61.84%)*	(-32.61%)*
Frontal Cortex	3.76	3.766	3.88	4.38
		(0.16%)*	(3.06%)*	(16.49%)*
Cerebral Cortex	3.67	2.879	4.06	4.14
		(-21.55%)*	(10.52%)*	(12.81%)8
Caudate nucleus	1.42	2.442	2.5	2.46
		(71.97%)*	(76.34%)*	(73.04%)*
Thalamus	5.84	6.094	4.6	21.4
		(4.37%)*	(-21.15%)*	(266.53%)
Hypothalamus	3.15	3.059	6.71	2.01
		(-2.89%)*	(112.86%)	(-36.19%)*
Hippocampus	0.71	0.591	0.49	0.59
		(-16.76%)*	(-31.40%)*	(-16.90%)*

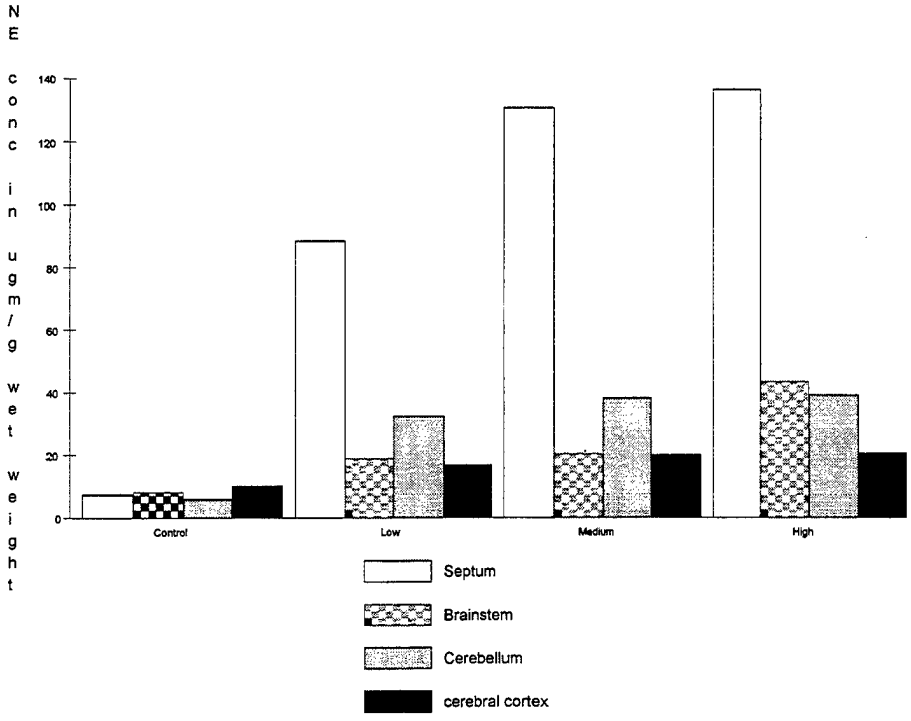
Note: Each value represents mean for 6 rats (conc., in μg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

TABLE 14. EFFECTS OF TRINITROBENZENE (LOW-, MID-, HIGH-DOSE) ON 5-HYDROXYINDOLACETIC ACID (5-HIAA) LEVELS IN NINE REGIONS OF BRAIN IN MALE RATS

Brain regions	5-HIAA conc in µg/g wet weight in control rats.	5-HIAA conc in µgm/g wet weight (low-dose) and %-change from control.	5-HIAA conc in µg/g wet weight in TNB (Mid-dose) and %-change from control.	5-HIAA conc in µg/g wet weight (high-dose) and %-change from control.
Septum	5.889	6.255	7.49	10.2
		(6.21%)*	(27.20%)*	(73.12%)*
Brainstem	2.62	2.021	2.37	3.71
		(-20.81%)*	(-7.42%)*	(43.75%)*
Cerebellum	4.271	2.016	1.6	2.82
		(-52.80%)*	(-62.64%)*	(-34.02%)*
Frontal Cortex	2.686	2.634	2.71	3.06
		(-1.94%)*	(0.90%)*	(14.05%)*
Cerebral Cortex	2.621		2.84	2.9
		(-23.20%)*	(8.20%)*	(10.44%)*
Caudate nucleus	1.014	1.708	1.75	1.72
		(68.36%)*	(72.64%)*	(69.41%)*
Thalamus	4.17	4.262	3.22	14.97
		(2.18%)*	(-22.80%)*	(258.84%)
Hypothalamus	2.25	2.139	4.69	1.44
		(-2.77%)*	(110.49%)	(-35.43%)*
Hippocampus	0.507	0.413	0.34	0.41
		(-18.51%)*	(-32.85%)*	(-18.64%)*

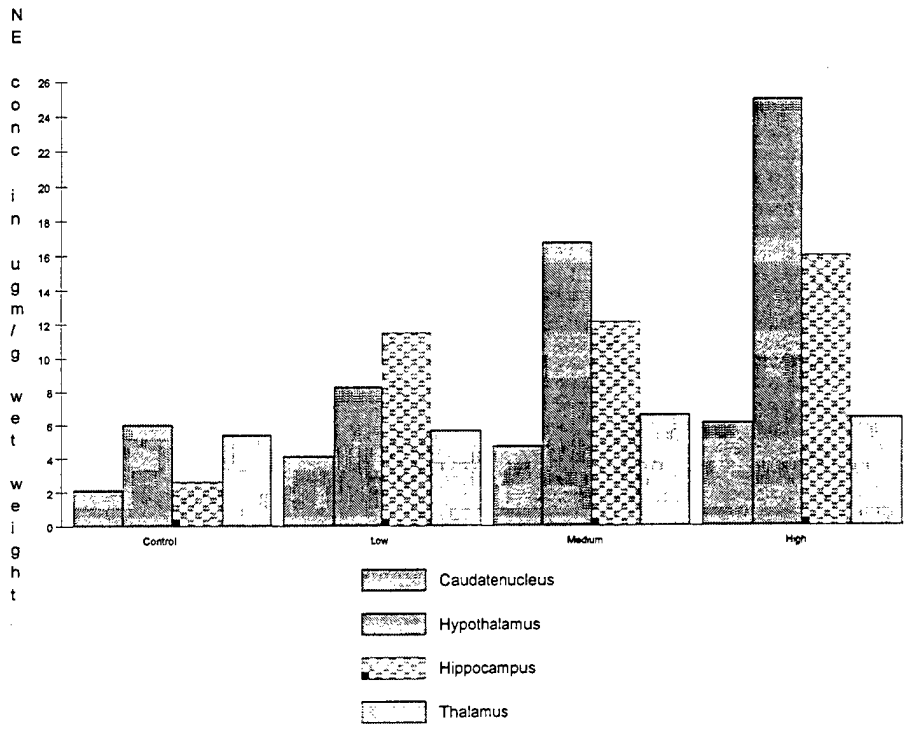
Note: Each value represents mean for 6 rats (conc., in µg/g wet weight). Standard error from the mean was less than 10. Significant difference ($p<0.01$) from control groups is indicated by %-change from control except for those with * (student's t-test for unpaired data.)

Norepinephrine(NE) Levels in Control and TNB Exposed Female Rats.
1. Septum 2. Brainstem 3. Cerebellum 4. Cerebral Cortex



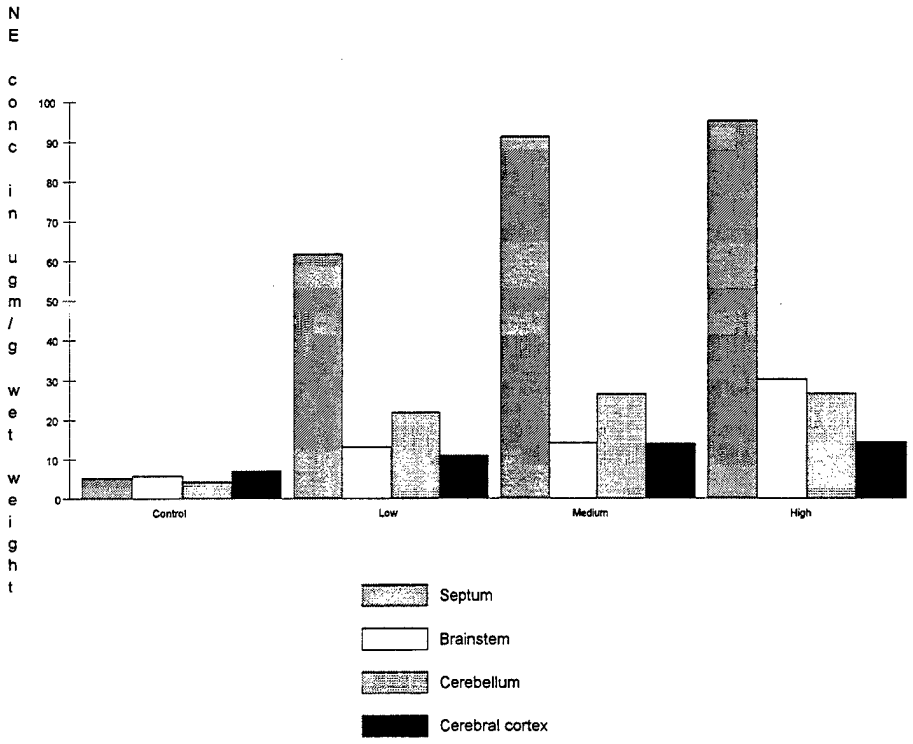
BARGRAPH A

Norepinephrine(NE) Levels in Control and TNB Exposed Female Rats.
1. Caudate Nucleus. 2. Hypothalamus 3. Hippocampus 4. Thalamus



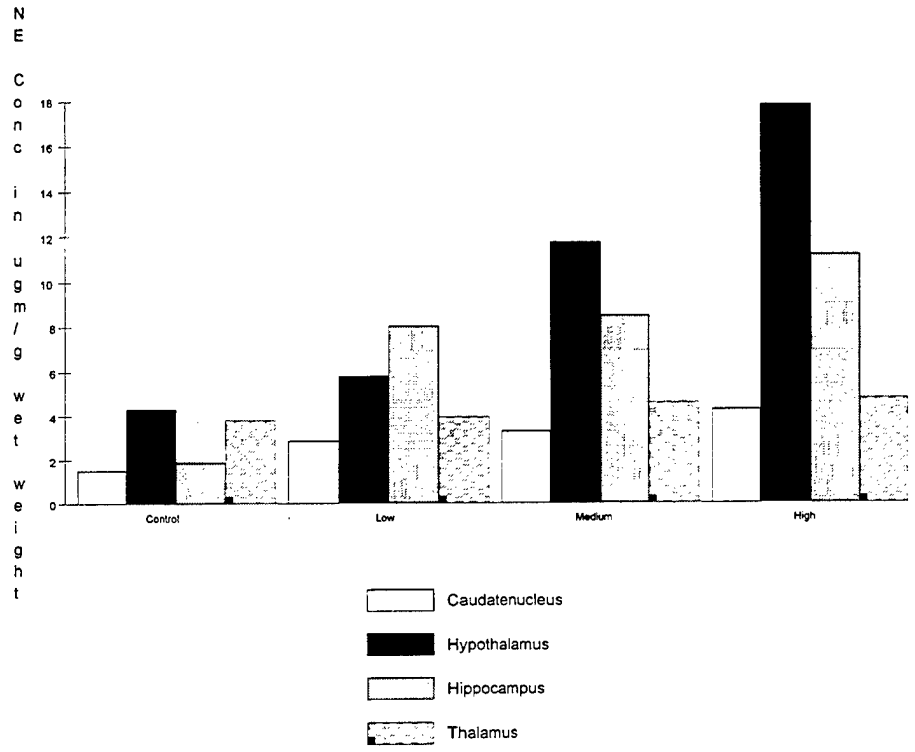
BARGRAPH B

Norepinephrine(NE) Levels in Control and TNB Exposed Male Rats.
1. Septum 2. Brainstem 3. Cerebellum 4. Cerebral Cortex



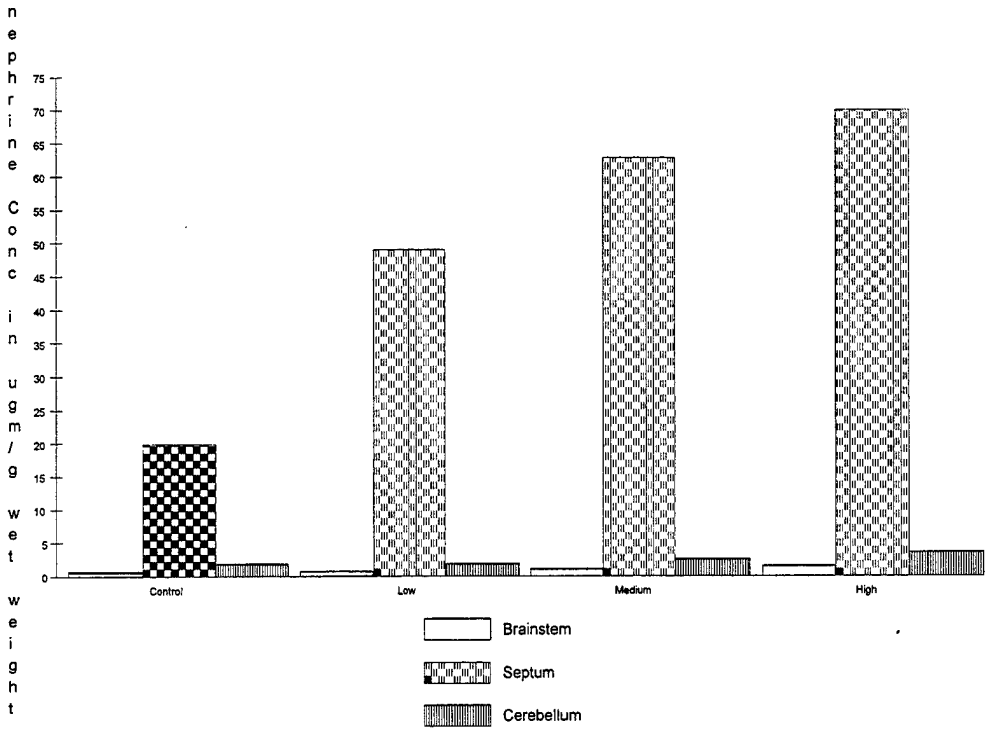
BARGRAPH C

Norepinephrine(NE) Levels in Control and TNB Exposed Male Rats.
1. Caudate nucleus 2. Hypothalamus 3. Hippocampus 4. Thalamus



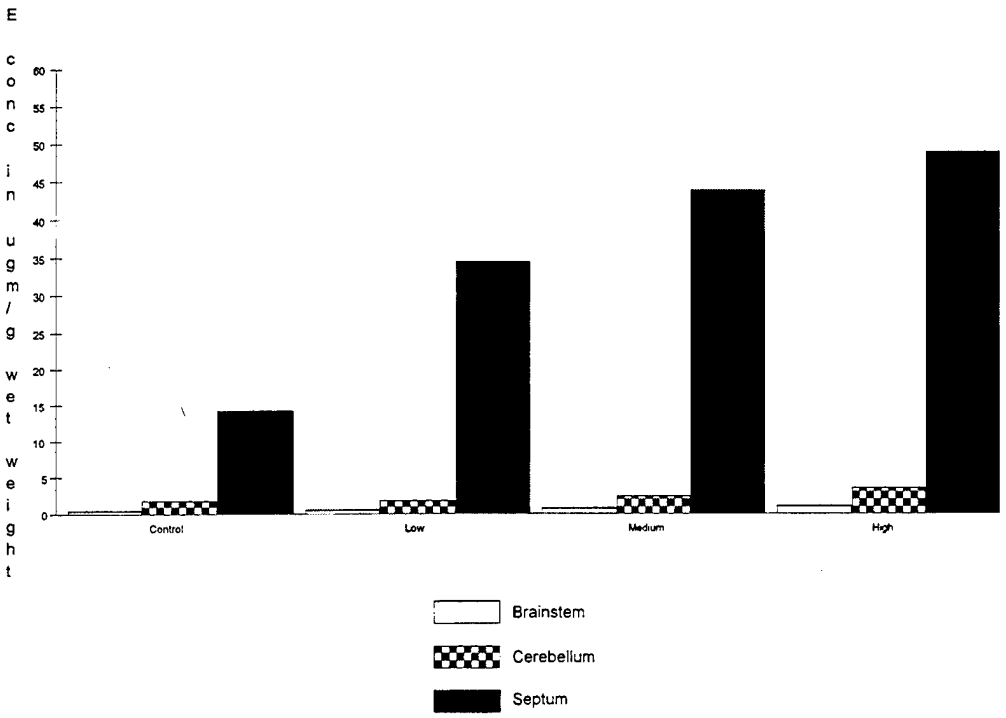
BARGRAPH D

Epinephrine Levels in Control and TNB Exposed Female Rats.
1. Brainstem 2. Septum 3. Cerebellum



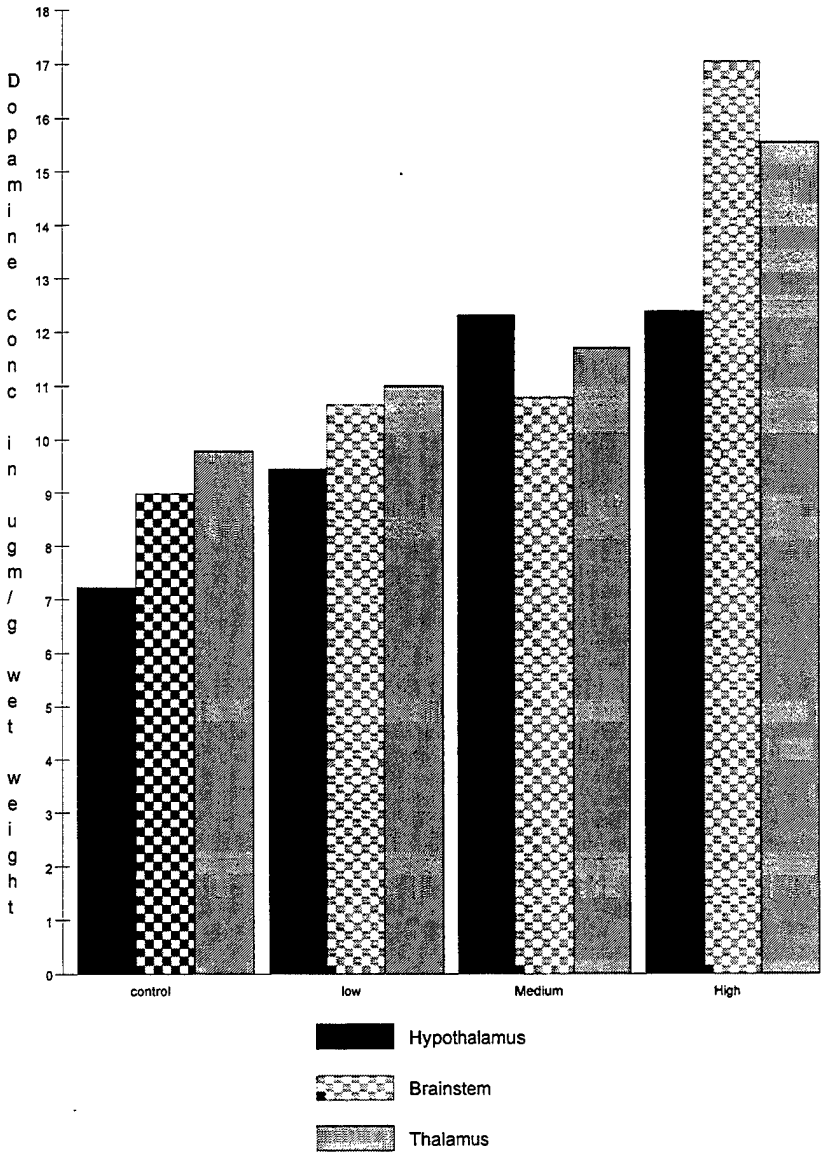
BARGRAPH E

Epinephrine(E) Levels in Control and TNB Exposed Male Rats.
1. Brainstem 2. Cerebellum 3. Septum



BARGRAPH F

Dopamine Levels in Control and TNB Exposed Female Rats.
1. Hypothalamus 2. Brainstem 3. Thalamus

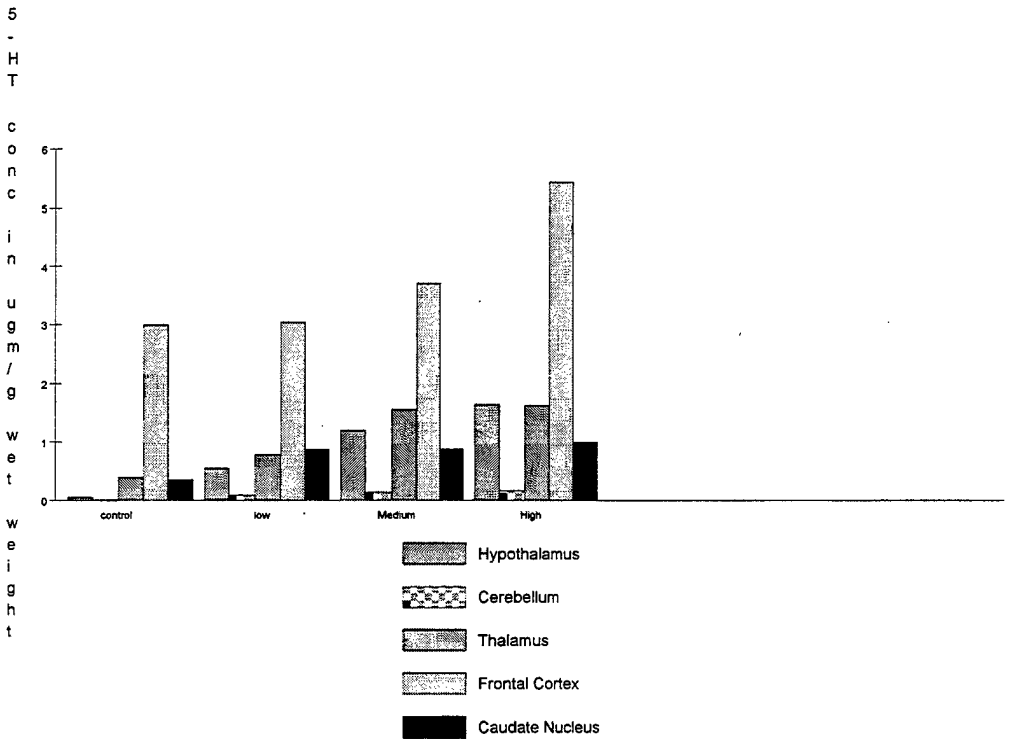


BARGRAPH G

Dopamine Levels in Control and TNB Exposed Male Rats.
1. Hypothalamus 2. Brain Stem 3. Thalamus

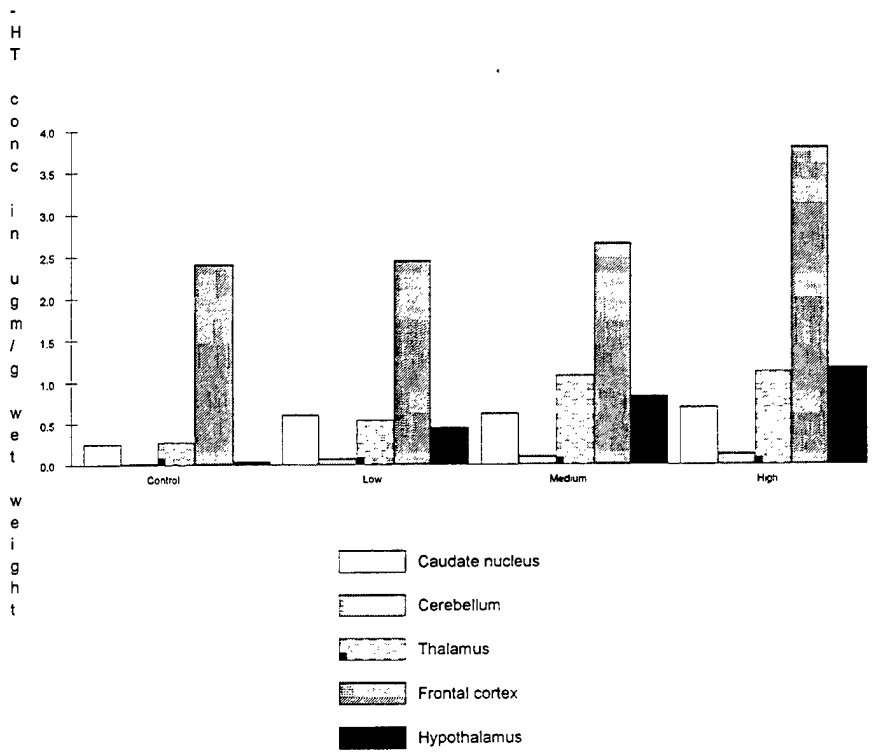
BARGRAPH H

5-Hydroxytriptamine(5-HT) Levels in Control and TNB Exposed Female Rats.
1. Hypothalamus 2. Cerebellum 3. Thalamus 4. Frontal Cortex 5. Caudate Nucleus



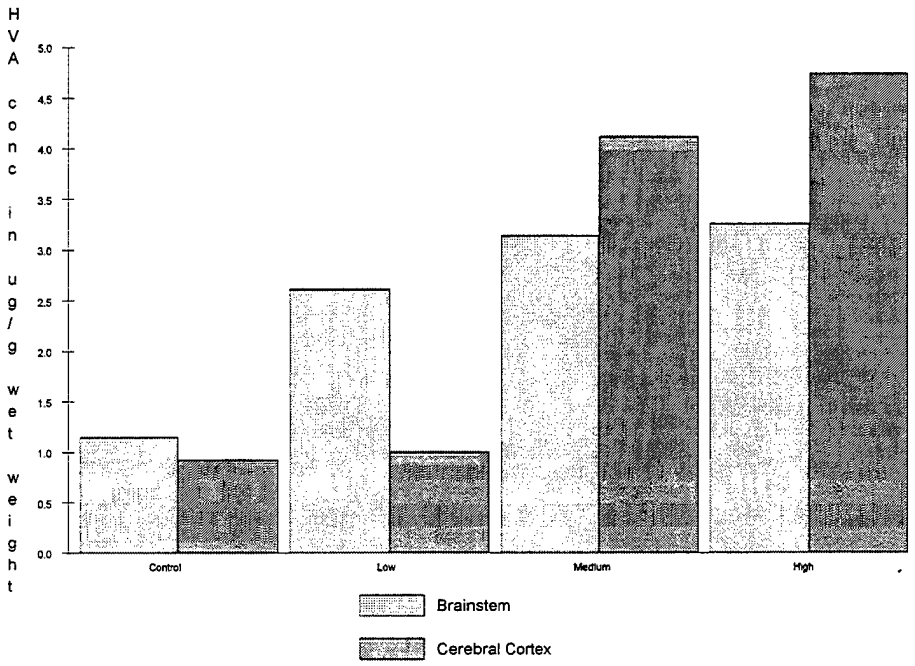
BARGRAPH I

5-Hydroxytriptamine(5-HT) Levels in Control and TNB Exposed Male Rats.
1. Caudate Nucleus 2. Cerebellum 3. Thalamus 4. Frontal Cortex 5. Hypothalamus



BARGRAPH J

Homovanillic Acid (HVA), Dopamine's Metabolite Levels in Control and TNB Exposed Rats.
1. Brainstem 2. Cerebral Cortex



BARGRAPH K

CONCLUSION

The environmental contaminant TNB induced signs of neurological disorders such as head tilting, loss of equilibrium and encephalitis. Lesions were observed in the medulla oblongata and cerebral peduncle. Neurotransmitter analysis in control and TNB-exposed rats showed a statistically significant increase in (a) norepinephrine levels in all the regions examined except the frontal cortex; (b) epinephrine levels in the brainstem, septum and cerebellum; (c) 5-HT level in the thalamus, hypothalamus, frontal cortex, caudate nucleus and cerebellum; and (d) dopamine levels in the thalamus, brainstem and hypothalamus. Neurotransmitter analysis also showed a decrease in dopamine levels in the caudate nucleus and septum in TNB-treated rats compared to control brain regions. Changes in the neurotransmitter levels in a specific region or regions may be one of the mechanism(s) responsible for the TNB-induced neurological disorder.

Along with the significant increase in NE and E levels, a significant increase in homovanillic acid (DA 's metabolite) was observed in TNB-exposed rats compared to control rats in the brainstem and cerebral cortex. Brain lesions were seen in the medulla oblongata (in brainstem) and cerebral peduncle (in cerebral cortex). Dopamine receptor binding studies and neurotoxic esterase studies in control and TNB-exposed rats' brainstem and cerebral cortex are recommended in the future to gain a better understanding of the possible biochemical mechanisms explaining TNB-induced brain lesions.

REFERENCES

1. M.G. Ryon, B.C. Pal, S.S. Talmage and R.H. Ross. 1984. Database assessment of the health and environmental effects of munitions production waste products. Final Report. ORNL-6018. (NTIS DE84-016512). Oak Ridge National Laboratory, TN, p. 3403.

2. U.S. EPA 1989. Health and environmental effects profile for 1,3,5-Trinitrobenzene. Environmental Criteria and Assessment office, Office of Health and Environmental Assessment, Cincinnati, OH, ECAO-CIN-G071.

3. C. Kim, M.B. Speisky and S.N. Kharouba. Rapid and sensitive method for measuring norepinephrine, dopamine, 5-hydroxytriptamine and their major metabolites in rat brain by high performance liquid chromatography. Journal of Chromatography,386 (1987) 25-35.

4. E.R. Kinkead, R.E. Wolfe, S.A Salins, C.D. Flemming, D.J. Caldwell, C.R. Miller and J.R. Latendresse. Range-finding study for a reproductive assessment of 1,3,5-Trinitrobenzene administered in the diet of Sprague-Dawley rats. Technical Report No. AL/OE-TR-1994-0072 (WRAIR/TR-94-0006). Armstrong Laboratory (AL/OET), Wright-Patterson AFB,OH-45433-7400.

5. E.R. Kinkead, R.E. Wolfe, C.D. Flemming; D.J. Caldwell, C.R. Miller and G.B Marit. Reproductive toxicity screen of 1,3,5-Trinitrobenzene administered in the diet of Sprague-Dawley rats. Technical Report No. AL/OE-TR-1994-0144 (WRAIR/TR-94-0016). Armstrong Laboratory (AL/OET), Wright-Patterson AFB,OH-45433-7400.